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CANCER ANTIGEN AND USE THEREOF

TECHNICAL FIELD

The present invention relates to a novel human cancer antigen that is useful for diagnosis of various types of cancers such as pancreatic cancer or colon cancer and for immunotherapy, and use thereof.

BACKGROUND ART

At present, cancer is the number one cause of death. Occurrence mechanisms, diagnostic methods, and therapeutic methods for cancer have been developed. However, a large number of advanced cancers have not yet been treated under the present circumstances. In order to improve the current situation, it is considered to be necessary to develop a novel early diagnostic method and therapeutic method.

Immunotherapy has long been anticipated as a method for treating cancers, and various attempts have been made regarding such therapy. However, sufficient antitumor effects have not yet been exhibited. Conventionally, immunotherapy for cancers had previously been centered on nonspecific immunotherapy. In recent years, however, it has been clarified that T cells play an important role in tumor rejection in living bodies. As a result, efforts are now focused on the isolation of a T cell-recognizing cancer antigen that is capable of inducing cytotoxic T lymphocytes (CTL) and the determination of an MHC class I-binding epitope.

To date, many cancer antigens have been isolated by the conventional cDNA expression cloning method, using CTL. This method requires the establishment of a cell line from tumor and the establishment of CTL. Thus, it is difficult to isolate a tumor antigen from carcinomas other than melanomas. In addition, in order to enhance the effects of immunotherapy, it is considered that a treatment method involving mixing many peptides is effective. In order to establish such a treatment method, it is necessary to isolate a large number of antigens. Thus, the conventional cDNA expression cloning method is problematic in that it takes enormous manpower and time to isolate even a single antigen.

In 1995, Pfreundschuh et al. in Germany and Old et al. in U.S.A. have reported the SEREX method, which detects a cancer antigen protein recognized by an antibody in the serum of a cancer patient (Serological Identification of Recombinant cDNA Expression Cloning; Proc. Natl. Acad. Sci. USA 92, 11810-11813, 1995). Many tumor antigens have been isolated by this method. Among antigens isolated by this method, antigens such as MAGE-1 or tyrosinase that induce CTL have also been included. Accordingly, it is pointed out that this method is also useful as a method for detecting an antigen recognized by cell-mediated immunity. Moreover, it has been reported that a cancer antigen recognized by the IgG antibody of a patient was isolated by the above-described method (Int. J. Cancer 72, 965-971, 1997; Cancer Res. 58, 1034-1041, 1998; Int. J. Cancer 29, 652-658, 1998; Int. J. Oncol. 14, 703-708, 1999; Cancer Res. 56, 4766-4772, 1996; and Hum. Mol. Genet 6, 33-39, 1997).

DISCLOSURE OF THE INVENTION

It is an object of the present invention to provide: a human pancreatic cancer antigen and/or a human colon cancer antigen that can be applied to the diagnosis and/or treatment of various types of cancers or tumors including pancreatic cancer and colon cancer as representative examples; a gene encoding the same; and an anti-cancer vaccine using the same.

That is to say, the present invention provides a cancer antigen comprising a protein of any of the following (A) or (B):

(A) a protein having the amino acid sequence shown in SEQ ID NO: 1; or

(B) a protein having an amino acid sequence comprising a substitution, deletion, insertion, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and also having immune-stimulating activity.

In another aspect, the present invention provides an immune-stimulating agent used for cancers, which comprises the aforementioned cancer antigen of the present invention.

In another aspect, the present invention provides a peptide comprising a portion of the aforementioned cancer antigen of the present invention and having immune-stimulating activity. The peptide of the present invention can preferably

activate cytotoxic T lymphocytes recognizing a cancer antigen protein. The peptide of the present invention preferably has the amino acid sequence shown in any one of SEQ ID NOS: 3 to 22.

In another aspect, the present invention provides a peptide, which has an amino acid sequence comprising a substitution, deletion, insertion, and/or addition of one or several amino acids with respect to the amino acid sequence shown in any one of SEQ ID NOS: 3 to 22, and also has immune-stimulating activity. The above-described peptide can preferably activate cytotoxic T lymphocytes which recognize a cancer antigen protein.

In another aspect, the present invention provides an immune-stimulating agent used for cancers, which comprises any one of the above-described peptides.

In another aspect, the present invention provides DNA encoding the aforementioned cancer antigen of the present invention.

In another aspect, the present invention provides DNA of any one of the following (a), (b), and (c):

(a) DNA having the nucleotide sequence shown in SEQ ID NO: 2;

(b) DNA hybridizing with the DNA having the nucleotide sequence shown in SEQ ID NO: 2 under stringent conditions, and encoding a protein having immune-stimulating activity; and

(c) DNA having a partial sequence of the DNA of (a) or (b) above, and encoding a protein having immune-stimulating activity.

In another aspect, the present invention provides an antibody against the aforementioned cancer antigen or peptide of the present invention.

In another aspect, the present invention provides helper T cells, cytotoxic T lymphocytes, or an immunocyte population comprising these cells, which are induced by *in vitro* stimulation using the aforementioned cancer antigen or peptide of the present invention, or a mixture thereof.

In another aspect, the present invention provides helper T cells, cytotoxic T lymphocytes, or an immunocyte population comprising these cells, which are induced by *in vitro* stimulation using the aforementioned cancer antigen or peptide of the present

invention, or a mixture thereof, and an immune activator. The immune activator is preferably a cell growth factor or cytokine.

In another aspect, the present invention provides a method for suppressing a tumor, which comprises introducing the above-described helper T cells, cytotoxic T lymphocytes, or an immunocyte population comprising these cells into a body. The above-described method is preferably used to prevent and/or treat cancers.

In another aspect, the present invention provides a cell culture solution used to produce the helper T cells or cytotoxic T lymphocytes of the present invention or an immunocyte population comprising these cells, which comprises the aforementioned cancer antigen or peptide of the present invention, or a mixture thereof.

In another aspect, the present invention provides a cell culture kit for producing the helper T cells or cytotoxic T lymphocytes of the present invention or an immunocyte population comprising these cells, which comprises the above-described cell culture solution and a cell culture vessel.

In another aspect, the present invention provides a cancer vaccine comprising the aforementioned cancer antigen and/or at least one type of peptide of the present invention. The above-described cancer vaccine preferably further comprises an adjuvant.

In another aspect, the present invention provides a cancer vaccine, which comprises the aforementioned DNA of the present invention, or recombinant virus or recombinant bacteria comprising the above-described DNA. The above-described cancer vaccine preferably further comprises an adjuvant.

In another aspect, the present invention provides a probe for diagnosing cancers, which comprises the aforementioned DNA of the present invention.

In another aspect, the present invention provides an agent for diagnosing cancers, which comprises the aforementioned cancer diagnostic probe and/or antibody of the present invention.

In another aspect, the present invention provides an agent for preventing and/or treating cancers, which comprises the aforementioned cancer antigen, peptide, antibody, and/or helper T cells, cytotoxic T lymphocytes, or an immunocyte population comprising these cells of the present invention.

In the present invention, cancer is preferably pancreatic cancer, colon cancer, brain tumor, malignant melanoma, chronic myelocytic leukemia, acute myelocytic leukemia, lymphoma, esophageal cancer, kidney cancer, prostatic cancer, lung cancer, breast cancer, stomach cancer, hepatic cancer, gallbladder cancer, testicular cancer, uterine cancer, ovarian cancer, thyroid cancer, bladder cancer, or sarcoma.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a microphotograph showing the results of an immunohistochemical analysis on hsp105 in pancreatic cancer. In the figure, the symbols have the following meanings:

A: a pancreatic cancer portion and a peripheral non-cancerous portion stained with hematoxylin and eosin, CA: a cancerous portion, NP: a non-cancerous portion

B: An hsp105 protein is highly expressed in cancer cells. It is weakly expressed also in a non-cancerous portion.

C: A non-cancerous portion is significantly expanded. An hsp105 protein is weakly expressed in the cytoplasm.

D: A cancerous portion is significantly expanded. An hsp105 protein is highly expressed mainly in the cytoplasm of cancer cells.

Figure 2 is a microphotograph showing the results of an immunohistochemical analysis on hsp105 in colon cancer. In the figure, the symbols have the following meanings:

A: a colon cancer portion and a peripheral non-cancerous portion stained with hematoxylin and eosin, CA: a cancerous portion, NP: a non-cancerous portion

B: An hsp105 protein is highly expressed in cancer cells. It is weakly expressed also in a non-cancerous portion.

C: A non-cancerous portion is significantly expanded. An hsp105 protein is weakly expressed in the cytoplasm.

D: A cancerous portion is significantly expanded. An hsp105 protein is highly expressed mainly in the cytoplasm of cancer cells. Also, the hsp105 protein is weakly expressed in the nucleus thereof.

Figure 3 is a graph showing the anticancer effects of an hsp105 DNA vaccine, an hsp105 peptide vaccine, and a control on mouse colon cancer cells Colon-26. A represents the area of a cancerous portion, B represents the ratio of mice wherein the cancer has developed, and C represents the ratio of surviving mice.

Figure 4 is a graph showing the results obtained by measuring by ^{51}Cr release assay, the cytotoxic activities on Colon-26 of various types of peptide vaccines derived from hsp105 proteins, or the cytotoxic activities of DNA vaccines encoding such hsp105 proteins.

Figure 5 shows the results of an immunohistochemical analysis on hsp105 in tissues.

Figure 6 shows the results obtained by analyzing the *in vivo* antitumor activity of a mouse CD4 positive helper T cell line induced by hsp105.

Figure 7 shows the results obtained by inducing by hsp105, the CD4 positive helper T cell line of a patient with colon cancer.

Figure 8 shows the results obtained by examining whether or not BALB/c mouse cytotoxic T lymphocytes (CTL) induced by an hsp105-derived peptide can reduce a tumor mass of the colon cancer cell line Colon-26 which highly expresses hsp105.

Figure 9 shows the results obtained by examining whether or not the cytotoxic T lymphocytes (CTL) of a colon cancer patient induced by an hsp105-derived peptide can reduce a tumor mass of the colon cancer cell line sw620, which highly expresses hsp105.

BEST MODE FOR CARRYING OUT THE INVENTION

The embodiments of the present invention will be described in detail below.

(1) The cancer antigen, peptide, and immune-stimulating agent against cancers according to the present invention

The cancer antigen of the present invention collected from pancreatic cancer or colon cancer is a protein of any of the following (A) or (B):

(A) a protein having the amino acid sequence shown in SEQ ID NO: 1 (hereinafter referred to as "hsp105"); or

(B) a protein having an amino acid sequence comprising a substitution, deletion, insertion, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and also having immune-stimulating activity.

The term "protein having immune-stimulating activity" is used in the present specification to mean a protein having activity of inducing an immune response such as generation of an antibody or cell-mediated immunity. Among them, a protein having T cell-stimulating activity of stimulating cytotoxic T lymphocytes (killer T cells/CTL) is particularly preferable.

hsp105 is a heat shock protein with a high molecular weight, which belongs to the hsp110/105 family, and is composed of hsp105α and 105β. 105α is a heat shock protein of 105 kDa, and is induced by various stresses. 105β is a protein generated by splicing of mRNA of 105α, and has a molecular weight smaller than that of 105α. hsp105 that is an antigen of pancreatic cancer or colon cancer of the present invention can be detected, for example, by the SEREX method as described later in examples in the present specification.

In the present invention, the scope of "one or several" in "an amino acid sequence comprising a substitution, deletion, insertion, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1" is not particularly limited. For example, it means 1 to 20, preferably 1 to 10, more preferably 1 to 7, further preferably 1 to 5, and particularly preferably 1 to 3 amino acids.

A method of obtaining or producing the cancer antigen protein of the present invention is not particularly limited. A naturally occurring protein, a chemically synthesized protein, or a recombinant protein produced by genetic engineering may be used. From the viewpoint that it can be produced in large volume by relatively easy operations, a recombinant protein is preferable.

When a naturally occurring protein is obtained, it can be isolated from cells or tissues expressing the protein by appropriate combined use of protein isolation and purification methods. When a chemically synthesized protein is obtained, the protein of the present invention can be synthesized by chemical synthesis methods such as the Fmoc method (fluorenylmethyloxycarbonyl method) or the tBoc method

(t-butyloxycarbonyl method). Moreover, the protein of the present invention can also be synthesized by using various types of commercially available peptide synthesizers.

When the cancer antigen protein of the present invention is produced in the form of a recombinant protein, it can be produced by obtaining DNA having a nucleotide sequence encoding the protein (e.g. the nucleotide sequence shown in SEQ ID NO: 2), a mutant thereof, or a homologue thereof, and introducing it into a preferred expression system.

Any expression vector can be used, as long as it can autonomously replicate in host cells or it can be incorporated into the chromosomes of host cells. An expression vector containing a promoter at a site that is capable of expressing the gene of the present invention, is used. A transformant having a gene encoding the protein of the present invention can be produced by introducing the aforementioned expression vector into a host. Such a host used herein may include bacteria, yeast, animal cells, and insect cells. In addition, an expression vector may be introduced into a host by known methods, depending on the type of the host.

In the present invention, the transformant having the gene of the present invention produced as described above is cultured, and the protein of the present invention is generated and accumulated in a culture product. Thereafter, the protein of the present invention is collected from the culture product, thereby isolating a recombinant protein.

When the transformant of the present invention is prokaryote such as *Escherichia coli* or eukaryote such as yeast, either a natural medium or a synthetic medium may be used as a medium in which these microorganisms are cultured, as long as it contains a carbon source, a nitrogen source, and inorganic salts that can be assimilated by the microorganisms, and the culture of the transformant can efficiently be carried out therein. In addition, culture may be carried out under conditions that are commonly used for culturing microorganisms. After completion of the culture, the protein of the present invention may be isolated and purified from the culture product of the transformant by common protein isolation and purification methods.

A protein having an amino acid sequence comprising a substitution, deletion, insertion, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, can appropriately be produced or obtained by persons

skilled in the art on the basis of the information regarding the nucleotide sequence shown in SEQ ID NO: 2, which is an example of the DNA sequence encoding the amino acid sequence shown in SEQ ID NO: 1.

That is to say, a gene (mutant gene) having a nucleotide sequence encoding a protein having an amino acid sequence comprising a substitution, deletion, insertion, and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, can be produced by any given methods that are known to persons skilled in the art, such as chemical synthesis, genetic engineering methods, or mutagenesis. Specifically, a mutation is introduced into DNA having the nucleotide sequence shown in SEQ ID NO: 2, so as to obtain mutant DNA.

For example, a method of allowing DNA to come into contact with an agent acting as a mutagen, a method of irradiating with ultraviolet rays, a genetic engineering method, and the like, can be applied to the DNA having the nucleotide sequence shown in SEQ ID NO: 2. Site-directed mutagenesis, one of the genetic engineering methods, is useful because it is capable of introducing a specific mutation into a specific site. Site-directed mutagenesis can be carried out according to the methods described in publications such as Molecular Cloning: A laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.,1989 (hereinafter abbreviated as Molecular Cloning 2nd Ed.); and Current Protocols in Molecular Biology, Supplements 1 to 38, John Wiley & Sons (1987-1997) (hereinafter abbreviated as Current Protocols in Molecular Biology).

The present invention also relates to a peptide that is a portion of the aforementioned protein of the present invention and has immune-stimulating activity. The peptide of the present invention preferably can activate cytotoxic T lymphocytes which recognize a cancer antigen protein. Specific examples of such a peptide may include those having any one of the following amino acid sequences:

Asn-Tyr-Gly-Ile-Tyr-Lys-Gln-Asp-Leu	(SEQ ID NO: 3)
Ala-Phe-Asn-Lys-Gly-Lys-Leu-Lys-Val-Leu	(SEQ ID NO: 4)
Lys-Tyr-Lys-Leu-Asp-Ala-Lys-Ser-Lys-Ile	(SEQ ID NO: 5)
Gln-Phe-Glu-Glu-Leu-Cys-Ala-Glu-Leu	(SEQ ID NO: 6)
Met-Tyr-Ile-Glu-Thr-Glu-Gly-Lys-Met-Ile	(SEQ ID NO: 7)

Thr-Phe-Leu-Arg-Arg-Gly-Pro-Phe-Glu-Leu	(SEQ ID NO: 8)
Glu-Tyr-Val-Tyr-Glu-Phe-Arg-Asp-Lys-Leu	(SEQ ID NO: 9)
His-Tyr-Ala-Lys-Ile-Ala-Ala-Asp-Phe	(SEQ ID NO: 10)
Lys-Tyr-Asn-His-Ile-Asp-Glu-Ser-Glu-Met	(SEQ ID NO: 11)
Ser-Leu-Asp-Glu-Lys-Pro-Arg-Ile-Val-Val	(SEQ ID NO: 12)
Arg-Leu-Tyr-Gln-Glu-Cys-Glu-Lys-Leu	(SEQ ID NO: 13)
Lys-Leu-Met-Ser-Ser-Asn-Ser-Thr-Asp-Leu	(SEQ ID NO: 14)
Leu-Met-Ser-Ser-Asn-Ser-Thr-Asp-Leu	(SEQ ID NO: 15)
Ser-Gln-Phe-Glu-Glu-Leu-Cys-Ala-Glu-Leu	(SEQ ID NO: 16)
Lys-Ile-Gly-Arg-Phe-Val-Val-Gln-Asn-Val	(SEQ ID NO: 17)
Tyr-Val-Tyr-Glu-Phe-Arg-Asp-Lys-Leu	(SEQ ID NO: 18)
Leu-Leu-Thr-Glu-Thr-Glu-Asp-Trp-Leu	(SEQ ID NO: 19)
Trp-Leu-Tyr-Glu-Glu-Gly-Glu-Asp-Gln-Ala	(SEQ ID NO: 20)
Glu-Leu-Met-Lys-Ile-Gly-Thr-Pro-Val	(SEQ ID NO: 21)
Val-Met-Asn-Ala-Gln-Ala-Lys-Lys-Ser-Leu	(SEQ ID NO: 22)

Moreover, peptides having an amino acid sequence comprising a substitution, deletion, insertion and/or addition of one or several amino acids with respect to the amino acid sequence shown in any one of the above SEQ ID NOS: 3 to 22, and having immune-stimulating activity, are also included in the scope of the present invention. A preferred example of such a peptide may be a peptide capable of activating cytotoxic T lymphocytes which recognize a cancer antigen protein.

In the present invention, the scope of "one or several" in "an amino acid sequence comprising a substitution, deletion, insertion and/or addition of one or several amino acids with respect to the amino acid sequence shown in any one of the above SEQ ID NOS: 3 to 22" is not particularly limited. The number of amino acids is, for example 1 to 5, preferably 1 to 4, more preferably 1 to 3, further preferably 1 or 2, and particularly preferably 1.

The peptide of the present invention can be synthesized by chemical synthesis methods such as the Fmoc method (fluorenylmethyloxycarbonyl method) or the tBoc method (t-butyloxycarbonyl method). Moreover, the peptide of the present invention

can also be synthesized using various types of commercially available peptide synthesizers.

The aforementioned cancer antigen protein and peptide of the present invention can induce immunity against cancers, as described later in examples. Accordingly, the present invention provides an immune-stimulating agent against cancers, which comprises the cancer antigen protein or peptide of the present invention.

The immune-stimulating agent against cancers of the present invention is used *in vitro* or *in vivo*, and preferably *in vitro*, so as to induce helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells, thereby providing immunity against cancers.

(2) DNA of the present invention

The DNA of the present invention encodes the cancer antigen protein of the present invention described in (1) above. It is preferably DNA of any one of the following (a), (b), and (c):

(a) DNA having the nucleotide sequence shown in SEQ ID NO: 2;

(b) DNA hybridizing with the DNA having the nucleotide sequence shown in SEQ ID NO: 2 under stringent conditions, and encoding a protein having immune-stimulating activity; and

(c) DNA having a partial sequence of the DNA according to (a) or (b) above, and encoding a protein having immune-stimulating activity.

The above term "DNA hybridizing with ... under stringent conditions" is used to mean the nucleotide sequence of DNA obtained by the colony hybridization method, the plaque hybridization method, or the Southern hybridization method, using DNA as a probe. For example, such DNA can be identified by hybridizing a filter, on which colony- or plaque-derived DNA or a DNA fragment thereof has been immobilized, at 65°C in the presence of 0.7 to 1.0 M NaCl, and then washing the filter at 65°C with a 0.1 to 2 x SSC solution (wherein 1 x SSC solution consists of 150 mM sodium chloride and 15 mM sodium citrate). Hybridization can be carried out by the method described in Molecular Cloning 2nd Ed.

DNA having a certain level of homology with the nucleotide sequence of DNA used as a probe is an example of the above DNA hybridizing under stringent conditions. Such DNA has homology of, for example 70% or more, preferably 80% or more, more preferably 90% or more, further preferably 93% or more, particularly preferably 95% or more, and most preferably 98% or more, with the DNA used as a probe.

A method of obtaining the DNA of the present invention is not particularly limited. Suitable probes or primers are prepared based on the information regarding the amino acid sequence and the nucleotide sequence shown in SEQ ID NOS: 1 and 2 in the sequence listing in the present specification, and the cDNA library of a human and the like is screened using such probes or primers, so as to isolate the DNA of the present invention. Such a cDNA library is preferably produced from a cell, organ, or tissue, which expresses the DNA of the present invention.

It is also possible to produce the DNA having the nucleotide sequence shown in SEQ ID NO: 2 by the PCR method. Using human chromosomal DNA or cDNA library as a template, PCR is carried out with a pair of primers that have been designed to amplify the nucleotide sequence shown in SEQ ID NO: 2. PCR reaction conditions can be determined as appropriate. For example, a reaction process consisting of 94°C and 30 seconds (denaturation), 55°C and 30 seconds to 1 minute (annealing), and 72°C and 2 minutes (elongation) is defined as 1 cycle. Such a reaction process is carried out 30 cycles, and thereafter, a reaction consisting of 72°C and 7 minutes is carried out. Thereafter, the amplified DNA fragment is cloned into a suitable vector capable of replicating in a host such as *Escherichia coli*.

The aforementioned preparation of probes or primers, construction of a cDNA library, screening of a cDNA library, and cloning of a gene of interest are already known to persons skilled in the art. These operations can be carried out according to the methods described in Molecular Cloning 2nd Ed., Current Protocols in Molecular Biology, and the like.

(3) Antibody of the present invention

The present invention further relates to an antibody recognizing a portion or the entire of the aforementioned protein or peptide of the present invention as an epitope

(antigen), and cytotoxic (killer) T lymphocytes (CTL) induced by *in vitro* stimulation using the above-described protein or peptide. In general, CTL exhibits stronger antitumor activity than an antibody.

The antibody of the present invention may be either a polyclonal antibody or a monoclonal antibody. It can be produced by common methods.

For example, a polyclonal antibody can be obtained by immunizing a mammal with the protein of the present invention as an antigen, collecting the blood from the mammal, and then separating and purifying an antibody from the collected blood. Examples of a mammal to be immunized may include a mouse, a hamster, a Guinea pig, a chicken, a rat, a rabbit, a dog, a goat, a sheep, and a bovine. The immunization method is known to those skilled in the art. For example, an antigen may be administered 2 or 3 times at intervals of 7 to 30 days. The dosage may be set at approximately 0.05 to 2 mg of antigen per administration. An administration route is not particularly limited. A suitable administration route can appropriately be selected from subcutaneous administration, intracutaneous administration, intraperitoneal administration, intravenous administration, and intramuscular administration. In addition, an antigen can be dissolved in a suitable buffer solution containing a commonly used adjuvant such as Freund's complete adjuvant or aluminum hydroxide, before use.

Such an immunized mammal has been bred for a certain period of time, and when its antibody titer begins to increase, a booster can be carried out using 100 μg to 1,000 μg of the antigen, for example. 1 or 2 months after the final administration, the blood is collected from the immunized mammal. The collected blood is then separated and purified by common methods including centrifugation, precipitation using ammonium sulfate or polyethylene glycol, or chromatography such as gel filtration chromatography, ion exchange chromatography, or affinity chromatography, so as to obtain a polyclonal antibody recognizing the protein of the present invention as a polyclonal antiserum.

On the other hand, a monoclonal antibody can be obtained by preparing hybridomas. Hybridomas can be obtained by cell fusion between antibody-generating cells and myeloma cells, for example. Hybridomas which generate the monoclonal antibody of the present invention can be obtained by the following cell fusion method.

Spleen cells, lymph node cells, B lymphocytes or the like collected from the immunized animal are used as antibody-generating cells. The protein of the present invention or a partial peptide thereof is used as an antigen. A mouse, a rat, or the like can be used as an animal to be immunized. The administration of an antigen to such an animal is carried out by common methods. For example, a suspension or emulsified liquid of an adjuvant such as Freund's complete adjuvant or Freund's incomplete adjuvant and the protein of the present invention used as an antigen is administered intravenously, subcutaneously, intracutaneously, or interperitoneally to an animal several times for immunization. For example, spleen cells are obtained from the thus immunized animal as antibody-generating cells, and the obtained cells are fused with myeloma cells by a known method (G. Kohler et al., Nature, 256, 495 (1975)), so as to produce hybridomas.

Examples of a myeloma cell line used for cell fusion may include mouse P3X63Ag8, mouse P3U1 line, and mouse Sp2/0 line. For cell fusion, fusion promoting agents such as polyethylene glycol or Sendai virus are used. For selection of hybridomas after completion of the cell fusion, hypoxanthine aminopterin thymidine (HAT) medium is used according to common methods. Hybridomas obtained by cell fusion are cloned by limiting dilution or the like. Thereafter, as necessary, screening is carried out by enzyme immunoassay using the protein of the present invention, so as to obtain a cell line generating a monoclonal antibody specifically recognizing the protein of the present invention.

In order to produce a monoclonal antibody of interest from the thus obtained hybridoma, the hybridoma may be cultured by the common cell culture method or ascites formation method, and the monoclonal antibody may be purified from the culture supernatant or ascites. The monoclonal antibody can be purified from the culture supernatant or ascites by common methods. For example, ammonium sulfate fractionation, gel filtration, ion exchange chromatography, affinity chromatography, and the like can appropriately be used in combination.

Moreover, fragments of the aforementioned antibody are also included in the scope of the present invention. Examples of such an antibody fragment may include a $F(ab')_2$ fragment and a Fab' fragment.

Furthermore, a labeled antibody obtained by labeling the aforementioned antibody is also included in the scope of the present invention. That is to say, the antibody of the present invention produced as described above can be labeled before use. The type of a substance used to label the antibody of the present invention and a labeling method are known to persons skilled in the art. Examples of such a labeling method may include: enzyme labeling with horseradish peroxidase or alkaline phosphatase; fluorescent labeling with FITC (fluorescein isothiocyanate) or TRITC (tetramethylrhodamine B isothiocyanate); labeling with color substances such as colloidal metal or colored latex; affinity labeling with biotin; and isotopic labeling with ^{125}I. The analysis or measurement of the protein of the present invention (that is a cancer antigen) with the labeled antibody of the present invention can be carried out according to methods widely known to those skilled in the art, such as the enzyme antibody technique, immunohistological staining, immunoblotting, the direct fluorescent antibody method, or the indirect fluorescent antibody method.

(4) Helper T cells, cytotoxic T lymphocytes, or immunocyte population containing these cells

The present invention further relates to helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells, which are induced by *in vitro* stimulation using the cancer antigen or peptide of the present invention or a mixture thereof. For example, when peripheral blood lymphocytes or tumor-infiltrating lymphocytes are stimulated *in vitro* with the protein or peptide of the present invention, tumor responsive activated T cells are induced. The activated T cells can effectively be used for adoptive immunotherapy. Moreover, the cancer antigen or peptide of the present invention is allowed to express in dendritic cells that are strong antigen presenting cells *in vivo* or *in vitro*, thereby conducting immune stimulation by administration of the dendritic cells wherein the antigen has been expressed.

Preferably, helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells are induced by *in vitro* stimulation using the cancer antigen or peptide of the present invention or a mixture thereof and an immune activator.

Examples of an immune activator used herein may include a cell growth factor and cytokine.

The helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells obtained as described above are transferred into a body to suppress tumor, thereby preventing and/or treating cancers.

Moreover, helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells, which can suppress tumor as described above, can be produced using the cancer antigen or peptide of the present invention or a mixture thereof. Accordingly, the present invention provides a cell culture solution containing the cancer antigen or peptide of the present invention or a mixture thereof. Using such a cell culture solution, the helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells, which can suppress tumor, can be produced. Further, the present invention provides a cell culture kit for producing the helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells, which comprises the aforementioned cell culture solution and a cell culture vessel.

(5) Cancer vaccine of the present invention

Since the DNA, cancer antigen, and peptide of the present invention can induce cancer cell-specific cytotoxic T lymphocytes, they are anticipated as therapeutic and/or preventive agents used for cancers. For example, BCG bacteria transformed by recombinant DNA obtained by incorporating the DNA of the present invention into a suitable vector, or viruses such as vaccinia virus into the genome of which the DNA of the present invention has been incorporated, can effectively be used as live vaccine for treating and/or preventing human cancers. The dosage and administration method of such a cancer vaccine are the same as those of ordinary vaccination or BCG vaccine.

Namely, the DNA of the present invention (as is, or in the form of plasmid DNA that is incorporated into an expression vector), and recombinant virus or recombinant bacteria containing the above DNA are administered as a cancer vaccine to mammals including a human, directly or in a state where they are dispersed in an adjuvant. Also, the peptide of the present invention can be administered thereto as a cancer vaccine, in a state where it is dispersed in an adjuvant.

Examples of an adjuvant used in the present invention may include Freund's incomplete adjuvant, BCG, trehalose dimycolate (TDM), lipopolysaccharide (LPS), alum adjuvant, and silica adjuvant. From the viewpoint of ability to induce antibody, Freund's incomplete adjuvant (FIA) is preferably used.

(6) Probe for diagnosing cancers, agent for diagnosing cancers, and preventive and/or therapeutic agent against cancers according to the present invention

The DNA of the present invention can be used as a diagnostic probe, in which DNAs of various types of human cancers are extracted and the homology between them is examined. Moreover, this probe and the above-described antibody can also be used as an agent for diagnosing cancers.

That is to say, the present invention relates to a probe for diagnosing cancers, which comprises an entire or a part of the antisense strand of DNA or RNA encoding the protein of the present invention. The present invention also relates to an agent for diagnosing cancers, which comprises the above-described probe for diagnosing cancers or an antibody reacting with the protein of the present invention. The probe for diagnosing cancers of the present invention is preferably the entire or a part of the antisense strand of DNA (cDNA) or RNA (cRNA) encoding the protein of the present invention, which preferably has a length that is long enough as a probe (at least 20 bases). For example, mRNA of the protein (cancer antigen) of the present invention obtained from an analyte is detected using the above-described antisense strand, thereby enabling the diagnosis of cancers. Examples of an analyte used for detection may include genomic DNA that can be obtained by biopsy of the cells of a subject, such as the blood, urine, saliva, or tissues; RNA; and cDNA, but examples are not limited thereto. When such an analyte is used, those amplified by PCR and the like may also be used.

The type of cancer is not particularly limited in the present specification. Specific examples of cancer may include pancreatic cancer, colon cancer, brain tumor, malignant melanoma, chronic myelocytic leukemia, acute myelocytic leukemia, lymphoma, esophageal cancer, kidney cancer, prostatic cancer, lung cancer, breast cancer, stomach cancer, hepatic cancer, gallbladder cancer, testicular cancer, uterine cancer, ovarian cancer, thyroid cancer, bladder cancer, and sarcoma.

The immune response of a cancer patient to cancer cells is unexpectedly active, and it is found that IgG antibodies are generated to various types of proteins. As described later in examples, hsp105 that is the antigen protein of the present invention is highly expressed specifically in pancreatic cancer, colon cancer, breast cancer, esophageal cancer, malignant lymphoma, pheochromocytoma, thyroid cancer, bladder cancer, and seminoma.

The protein or peptide of the present invention can induce cancer cell-specific cytotoxic T lymphocytes as T cell epitopes. Thus, it is useful as a preventive and/or therapeutic agent used for human cancers. In addition, the antibody of the present invention is also effective as a preventive and/or therapeutic agent used for human cancers, as long as it can inhibit the activity of the protein of the present invention that is a cancer antigen. As a practical usage, the protein, peptide, or antibody of the present invention may be administered directly, or together with a pharmaceutically acceptable carrier and/or diluent, or also together with the below-mentioned assistant agents, as necessary, so as to create an injection. Otherwise, it may also be administered by percutaneous absorption via the mucosa according to a method such as spraying. The term "carrier" is used herein to mean, for example, human serum albumin. Examples of a diluent may include PBS and distilled water.

As a dosage, the cancer antigen, peptide, or antibody of the present invention may be administered within a range between 0.01 mg and 100 mg per once per adult. However, a dosage is not limited to the above range. Also, the dosage form is not particularly limited. A freeze-dried product, or granules produced by adding an excipient such as sugar to the cancer antigen, peptide, or antibody of the present invention, may also be used.

Examples of an assistant agent that can be added to the agent of the present invention to enhance the activity of inducing cytotoxic T lymphocytes may include fungal components of BCG bacteria or the like, ISCOM described in Nature, vol. 344, p. 873 (1990), QS-21 of saponins described in J. Immunol. vol. 148, p.1438 (1992), liposome, and aluminum hydroxide. In addition, immune activators such as lenthinan, schizophyllan, or Picibanil may also be used as assistant agents. Moreover, cytokines

which promote the growth or differentiation of T cells, such as IL-2, IL-4, IL-12, IL-1, IL-6, or TNF, may also be used as assistant agents.

Furthermore, the above-described antigen peptide is added to cells collected from a patient or cells having the same HLA haplotype in a test tube, so as to allow the cells to present an antigen. Thereafter, it is administered into the blood vessel of the patient, so that cytotoxic T lymphocytes can effectively be induced in the patient body. Moreover, the above-described peptide is added to the peripheral blood lymphocytes of a patient, and it is then cultured in a test tube, so that cytotoxic T lymphocytes can be induced in the test tube and then returned to the blood vessel of the patient. Such a treatment involving cell transfer has already been conducted as a cancer treatment, and thus, it has been well known to persons skilled in the art.

A target antigen in the specific antitumor immunotherapy is required to be an antigen recognized by cytotoxic T lymphocytes (killer T cells/CTL). The antigen of the present invention has increased *in vitro* killer T cell-stimulating activity on HLA-A24 that is widely found in Japanese, or on HLA-A2 that is widely found over the world. Thus, the injection of the antigen of the present invention into a body induces the activation of CTL, and as a result, it can be anticipated that antitumor effects be obtained. Moreover, when lymphocytes are stimulated with the antigen of the present invention *in vitro*, activated T cells are induced. The thus activated T cells are injected into an affected area. Thus, this method can effectively be used as an adoptive immunotherapy.

EXAMPLES

The antigen of the present invention, the production method thereof, and the effects thereof will be described in the following examples. However, these examples are not intended to limit the scope of the present invention.

Example 1

<Collection of serum>

The serum was collected from a patient with pancreatic cancer. The collected serum was conserved at -80°C. From this serum sample, an antibody reacting with *Escherichia coli* and λ phage was eliminated by using a column that was filled with

dissolved matter of *Escherichia coli* and phage and sepharose 4B. Thereafter, the resultant serum was 100 to 800 times diluted and then used.

<cDNA library and production of protein>

A phage cDNA library produced by inserting cDNA of a pancreatic cancer cell line CFPAC-1 into a λZAP express vector was purchased from Stratagene, La Jolla, CA. *Escherichia coli* was infected with this λ phage cDNA library, and it was then cultured on NZY plate medium at 42°C for 6 hours, so as to produce plaques. Thereafter, the plate was covered with a nitrocellulose membrane into which isopropyl β-D-thiogalactoside (IPTG) had been infiltrated at 37°C for 3 hours, so as to produce a protein encoded by the cDNA that had been incorporated into λ phage in the plaques.

<Immunoscreening>

The protein produced by the aforementioned method was transferred into a nitrocellulose membrane. After blocking, the nitrocellulose was washed, and it was then allowed to react with the above-described serum at 4°C for 15 hours. After washing, a horseradish peroxidase (HRP)-labeled mouse anti-human IgG antibody was used as a secondary antibody, and it was allowed to react with the membrane. After washing, chemoluminescence was detected on an X-ray film, and it was compared with the plate on a photograph, so that positive plaques were picked up together with the peripheral negative plaques. Plaques corresponding to positive sites in a color reaction were collected from a 15-cm NZY agarose plate. The collected plaques were then dissolved in an SM buffer solution (100 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris-HCl, and 0.01% gelatin; pH 7.5). The color reaction positive plaques were subjected to a second screening and a third screening by the same above method, until they became a single colony, thereby obtaining a single phage clone with which an IgG antibody in the serum reacts. By the above-described method, 63 positive clones were isolated from cDNA derived from the pancreatic cancer cell line.

<Homology search of isolated antigen gene>

Insert DNA was amplified by the PCR method, and it was used for the subsequent analysis. The obtained PCR product was sequenced using Big Dye DNA sequencing kit (PE Biosystems, CA), so as to determine a nucleotide sequence thereof. Using homology search program BLAST (Basic Local Alignment Search Tool), each of the thus

determined nucleotide sequences of 63 types of genes was compared with the gene information registered in the NCBI databank.

<hsp105>

As a result, 18 positive clones shown in Table 1 were found. One of the positive clones was hsp105.

TABLE 1

Genes Isolated by SEREX of a Pancreatic Ductal Adenocarcinoma Cell Line

Gene designation	Gene/sequence identity	SEREX database search[a]
KM-PA-1	apg-2 (heat shock protein 110 family)	NGO-St-81, NY-CO-40, NY-CO-32
KM-PA-2	EST (KIAA0124)	—
KM-PA-3	β-actin	—
KM-PA-4	coactosin-like protein (CLP)	—
KM-PA-5	HALPHA44 (alpha-tubulin)	—
KM-PA-6	unknown	—
KM-PA-7	CDC-like kinase (CLK3)	—
KM-PA-8	cytokeratin 18	—
KM-PA-9	polyA binding protein	—
KM-PA-10	very-long-chain-acyl-CoA-dehydrogenase (VLCAD)	—
KM-PA-11	unknown	—
KM-PA-12	HLA-Cw heavy chain (MHC Class I)	LONY-BR-26
KM-PA-13	unknown	—
KM-PA-14	CGI 55 protein	—
KM-PA-15	glycosylation-inhibiting factor (GIF)	Mz19-16a, Hom-HD1-21
KM-PA-16	unknown	NGO-St-95, NGO-St-103
KM-PA-17	DNA binding protein A (dbpA)	—
KM-PA-18	heat shock protein 105 (KIAA0201)	NY-CO-25

[a] Dash means no strong homology.

<Confirmation of expression of hsp105>

The presence or absence of the expression of an hsp105 protein was immunohistochemically analyzed in various types of cancer tissues and in normal tissues. As a result, it was found that hsp105 is expressed in pancreatic cancer tissues and in colon cancer tissues, as shown in Figures 1 and 2.

Example 2

<Peptide constituting hsp105>

A motif binding to HLA-A24, for which 60% of Japanese people get positive, is almost identical to a motif, to which K^d of a BALB/c mouse binds. A peptide that is shared by human hsp105 and mouse hsp105 and is predicted to bind to both HLA-A24 and K^d is selected from the sequence of hsp105, using HLA-peptide binding prediction (http://bimas/dcrt.nih.gov/molbio/hla_bind/). Nine types of peptides consisting of 9 or 10 amino acids were synthesized by the Fmoc/PyBOP method. The sequences of the peptides and the estimated binding values to K^d are shown in Table 2.

Table 2

hsp105-derived peptides

hsp105-derived peptides			
No.	Position	Sequence	Binding Score
1	hsp105 180-188	NYGIYKQDL	2400
2	hsp105 214-223	AFNKGKLKVL	960
3	hsp105 251-260	KYKLDAKSKI	2880
4	hsp105 305-313	QFEELCAEL	1382
5	hsp105 433-442	TFLRRGPFEL	1920
6	hsp105 570-579	MYIETEGKMI	4800
7	hsp105 597-606	ECVYEFRDKL	80
8	hsp105 682-690	HYAKIAADF	60
9	hsp105 696-705	KYNHIDESEM	432

Example 3

<DNA vaccine>

Plasmid DNA produced by incorporating mouse hsp105 cDNA into an expression vector pCAGGS was adjusted to be a suitable concentration. It was then used as a vaccine for the following performance evaluation test. With regard to this mouse hsp105-pCAGGS DNA vaccine, *Escherichia coli* was cultured, and thereafter, plasmid DNA was extracted from the *Escherichia coli* and purified, so as to produce the vaccine in large scale.

<Anti-cancer effects of peptide vaccine and DNA vaccine>

The following samples were injected into the muscle of each BALB/c mouse: (1) a normal saline solution, (2) only a vector, (3) a hsp105 cDNA vector, (4) only an adjuvant, (5) an adjuvant + a peptide. Thereafter, a colon cancer cell line Colon-26 derived from a syngeneic mouse, that highly expresses hsp105, was subcutaneously transplanted into the back of the mouse. Thereafter, the development of the cancer in the mice was evaluated in the following points: (1) the area of a cancerous portion, (2) the ratio of mice in which the cancer developed, and (3) the ratio of surviving mice. The results are shown in Figures 3A, 3B, and 3C.

As shown in Figures 3A, 3B, and 3C, when 3 x 10^4 cells of Colon-26 were transplanted, until 13 days after the immunization, a tumor developed in all the 5 mice immunized with a normal saline solution and in all the 5 mice immunized with only pCAGGS. On the other hand, in the case of 5 mice immunized with hsp105-DNA vaccine, a tumor developed in one mouse 20 days after the immunization and in another mouse 24 days after the immunization. However, the remaining 3 mice completely rejected the development of a tumor. In the case of the adjuvant administration group, a tumor developed in all the 5 mice until 24 days after the immunization. There were observed significant differences between the DNA vaccine-, peptide vaccine-, and adjuvant-administration groups, and the normal saline solution- and vector-administration groups (Figure 3B). The same results were obtained regarding the mean tumor area (Figure 3A).

From these results, it is clear that the peptide vaccine and the DNA vaccine have the effects as anticancer agents. Considering a survival curve, 2 out of the 5 mice still survived 45 days after the immunization in the normal saline solution-, vector-, and adjuvant-administration groups. Moreover, all the 5 mice survived in the DNA

vaccine-administration group. The DNA vaccine group had significant differences from the other 4 groups. The peptide vaccine group had a significantly longer survival period than those of the normal saline solution-, vector-, and adjuvant-administration groups (Figure 3C). Furthermore, the mice that rejected the development of a tumor were pathologically observed, and it was confirmed that there were no damage on normal organs and that a large number of inflammatory cells filtrated into sites that rejected the development of a tumor.

<Determination of CTL epitope peptide of hsp105>

In order to identify a CTL epitope peptide, pancreatic cells were recovered from the mice, on which the DNA vaccine-peptide vaccine had worked. The recovered cells were stimulated once with the 9 types of peptides shown in Table 2, and the cytotoxic activity on Colon-26 was analyzed by ^{51}Cr release assay. As a result, it was found that among the above-described 9 types of peptides, the following 5 types of peptides 1, 2, 3, 4, and 5 are useful (Figure 4).

Asn-Tyr-Gly-Ile-Tyr-Lys-Gln-Asp-Leu (1)

Ala-Phe-Asn-Lys-Gly-Lys-Leu-Lys-Val-Leu (2)

Lys-Tyr-Lys-Leu-Asp-Ala-Lys-Ser-Lys-Ile (3)

Gln-Phe-Glu-Glu-Leu-Cys-Ala-Glu-Leu (4)

Met-Tyr-Ile-Glu-Thr-Glu-Gly-Lys-Met-Ile (5)

<Agent for diagnosing cancers>

Using an hsp105 antibody, the pathological diagnosis of the following cancers can be conducted: pancreatic cancer, colon cancer, brain tumor, malignant melanoma, chronic myelocytic leukemia, acute myelocytic leukemia, lymphoma, esophageal cancer, kidney cancer, prostatic cancer, lung cancer, breast cancer, stomach cancer, hepatic cancer, gallbladder cancer, testicular cancer, uterine cancer, ovarian cancer, thyroid cancer, bladder cancer, and sarcoma.

<CTL cancer therapeutic agent>

In the case of mice, it was clarified that killer T cells recognizing hsp105 and/or a peptide constituting the hsp105 as an antigen do not impair normal cells and have cytotoxic activity only on mouse colon cancer. Thus, there is a possibility that CTL can

be used as a cancer therapeutic or preventive agent with few side effects in human pancreatic and colon cancers wherein hsp105 is highly expressed.

Example 4: Identification of CTL epitope peptide of HLA-A24 of hsp105 in human

In order to determine the CTL epitope peptide of HLA-A24 of hsp105 in human, the peripheral blood lymphocytes collected from two colon cancer patients with HLA-A24 were stimulated 4 times with 9 types of peptides shown in Table 3. Thereafter, the cytotoxic activity on a human colon cancer cell line sw620, which highly expresses hsp105 and also expresses HLA-A24, was analyzed by ^{51}Cr release assay.

Specifically, monocytes were separated from the peripheral blood. Two millions of monocytes per well on a 24-well plate were cultured in 2 ml of a culture solution containing 10% autoserum, IL-2 (100 IU/ml), and 10 μM each peptide for 1 week. Thereafter, every week, the above culture product was stimulated with 200,000 dendritic cells (DC), which had been induced over 1 week, pulsed with 10 mM the above peptide and exposed to radioactive rays, repeatedly 3 times. 6 days later, the cytotoxic activity thereof was analyzed. Herein, two millions of monocytes were cultured in the presence of GM-CSF (100 ng/ml) and IL-4 (100 U/ml) for 5 days, and TNF-α (20 ng/ml) was further added thereto, followed by culture for 2 days. The obtained culture product was used as DC.

CTL which impairs C1RA2402 cells stimulated with the above peptide rather than those that were not stimulated with the above peptide was defined as peptide-specific positive. The results are shown in Table 3. The boldface figures in Table 3 indicate that CTL that is specific for the peptide and has cancer cell cytotoxicity can be induced.

Table 3: Peptides which can induce peptide-specific and cancer cell cytotoxic killer T cell by stimulating human peripheral blood lymphocytes of HLA-A24

hsp105-derived peptide	Position	Sequence	HLA-A2402-binding score	each peptide-induced CTLs from Pt 1 (HLA-A2402/) % Lysis to sw620 (HLA-A 0201/2402)	C1RA2402	C1RA2402 peptide 10μM	each peptide-induced CTLs from Pt 2 (HLA-A2402/) % Lysis to sw620 (HLA-A0201)	C1RA2402	C1RA2402 peptide 10μM
A24-1	180-188	NYGIYKQDL	240	16	42	31	32	13	25
A24-2	214-223	AFNKGKLKVL	30	0	42	49	40	28	54
A24-3	251-260	KYKLDAKSKI	110	50	29	46	21	33	44
A24-4	305-313	QFEELCAEL	48	48	22	43	16	40	38
A24-5	433-442	TFLRRGPFEL	33	53	33	33	26	33	46
A24-6	613-622	MYIETEGKMI	90	49	22	47	29	28	52
A24-7	640-649	EYVYEFRDKL	330	40	22	45	8	26	31
A24-8	725-733	HYAKIAADF	140	41	25	37	66	28	43
A24-9	739-748	KYNHIDESEM	83	19	36	43	33	24	45

Example 5: Identification of CTL epitope peptide of HLA-A2 of hsp105 in human

In order to determine the CTL epitope peptide of HLA-A2 of hsp105 in human, the peripheral blood lymphocytes of a colon cancer patient with HLA-A2 and those of a healthy subject with HLA-2 were stimulated 4 times with 30 types of peptides shown in Table 4. Thereafter, the cytotoxic activity on a human colon cancer cell line sw620, which highly expresses hsp105 and also expresses HLA-A2, was analyzed. Specific experimental methods were the same as those in Example 4.

In addition, sw620 cells, the expression level of hsp105 of which was reduced by RNAi, was defined as sw620 hsp105-RNAi cells. If CTL did not impair the sw620 hsp105-RNAi cells, then it was judged that it had cytotoxic activity specific for hsp105. Moreover, CTL impairing sw620 hsp105-RNAi cells stimulated with the above peptide rather than those that were not stimulated with the above peptide was defined as peptide-specific positive. The results are shown in Table 4. The boldface figures in Table 4 indicate that CTL that is specific for the peptide and has cancer cell cytotoxicity can be induced.

Table 4: Peptides which can induce peptide-specific and cancer cell cytotoxic killer T cell by stimulating human peripheral blood lymphocytes of HLA-A2

hsp105-derived peptide	Position	Sequence	HLA-A0201-binding score	each peptide-induced CTLs from Pt 1 (HLA-A0207/3301)			each peptide-induced CTLs from HD 1 (HLA-A0201/0207)		
				% Lysis to			% Lysis to		
				sw620 (HLA-A0201)	sw620 hsp105-RNAi	sw620 hsp105-RNAi peptide 10μM	sw620 (HLA-A0201)	sw620 hsp105-RNAi	sw620 hsp105-RNAi peptide 10μM
A2-1	86-94	NLSYDLVPL	49	5	68	56	-	-	-
A2-2	103-111	VMYMGEEHL	41	20	41	36	-	-	-
A2-3	105-114	YMGEEHLFSV	12637	5	0	0	-	-	-
A2-4	120-128	MLLTKLKET	107	0	0	1	6	35	3
A2-5	141-149	VISVPSFFT	55	4	0	5	-	-	-
A2-6	155-163	SVLDAAQIV	37	5	7	18	4	0	13
A2-7	169-177	RLMNDMTAV	591	4	0	8	2	29	32
A2-8	190-199	SLDEKPRIVV	46	30	18	0	26	9	40
A2-9	202-210	DMGHSAFQV	21	26	0	3	-	-	-
A2-10	222-231	VLGTAFDPFL	759	0	29	20	2	0	0
A2-11	265-273	RLYQECEKL	33	18	0	28	15	0	17
A2-12	275-284	KLMSSNSTDL	276	10	1	13	10	28	58
A2-13	276-284	LMSSNSTDL	26	11	0	21	11	0	14
A2-14	300-309	KMNRSQFEEL	50	11	0	0	44	61	9
A2-15	304-313	SQFEELCAEL	32	12	0	4	21	0	9
A2-16	313-321	LLQKIEVPL	36	10	21	8	-	-	-
A2-17	323-332	SLLEQTHLKV	1055	1	76	34	32	0	0
A2-18	381-389	AILSPAFKV	205	50	0	0	22	28	9
A2-19	434-422	FLRRGPFEL	43	8	39	3	-	-	-
A2-20	458-467	KIGRFVVQNV	76	24	0	9	32	9	4
A2-21	601-610	NLVWQLGKDL	21	7	0	4	5	0	4
A2-22	602-610	LVWQLGKDL	26	19	0	3	-	-	-
A2-23	641-649	YVYEFRDKL	210	26	2	13	0	9	23
A2-24	648-657	KLCGPYEKFI	200	9	0	0	42	0	9
A2-25	668-676	LLTETEDWL	401	32	0	27	23	42	27
A2-26	675-684	WLYEEGEDQA	146	18	0	41	11	21	3
A2-27	694-702	ELMKIGTPV	19	14	0	13	22	0	0
A2-28	714-723	KMFEELGQRL	819	11	2	0	5	0	0
A2-29	757-765	EVMEWMNNV	15	1	0	0	-	-	-
A2-30	765-774	VMNAQAKKSL	26	0	0	11	26	0	12

Example 6: Results of immunohistochemical analysis of hsp105 in tissues

The expression of hsp105 in various tissues was immunohistochemically analyzed. Specifically, thin sections with a size of 3 mm were prepared from blocks obtained by immobilizing various tissues with formalin and embedding them in paraffin. Thereafter, using VECTOR stain ABC-PO (rabbit IgG) kit (Vector Laboratories, Inc. Burlingame, CA), these sections were subjected to immunohistochemical analysis by the ABC method (avidin-biotin complex immune peroxidase technique). Rabbit polyclonal anti-human HSP105 Ab (SANTACRUZ, Santa Cruz, CA) was purchased, and it was then 200 times diluted. The obtained product was used as a primary antibody.

Figure 5 is a microphotograph showing the results of the above immunohistochemical analysis. In Figure 5, symbols have the following meanings. A: colon cancer, B: colon cancer in colon polyp, C: liver metastasis of colon cancer, D: pancreatic cancer, E: insulinoma, F: papillary adenocarcinoma in breast cancer, G: scirrhus cancer in breast cancer, H: esophageal cancer, I: thyroid cancer, J: gastric malignant lymphoma, K: pheochromocytoma, L: bladder cancer, M: testis, and N: seminoma. As is clear from the results shown in Figure 5, a high level of expression of hsp105 was observed in A, B, C, D, E, F, H, I, J, K, L, M, and N, that is, tumors other than G, and also in the testis.

Example 7: *In vivo* antitumor activity of mouse CD4 positive helper T cell line induced by hsp105

The spleen of a BALB/c mouse was collected and ground to separate spleen cells. 200,000 spleen cells per well on a 96-well flat plate were cultured in 200 μl of a culture solution containing IL-2 (100 IU/ml) and a 2 μg/ml recombinant hsp105 protein for 1 week. Thereafter, every week, the culture product was repeatedly stimulated with 200,000 spleen cells, which had been pulsed with a 2 μg/ml recombinant hsp105 protein and then exposed to radioactive rays, so as to establish multiple CD4 positive helper T cell lines (Th). The expression of CD4 and CD8 on the surface of cells was confirmed by performing immunofluorescent staining with Monoclonal Antibody MOUSE CD4-FITC, CD8-FITC (IMMUNOTECH, Marseille, France), and then analyzed with FACS (Figure 6A). It was examined by the intake of [^{3}H] thymidine, whether or not Th

specifically reacts with the hsp105 protein and grows. Specifically, 150,000 spleen cells were placed in each well of a 96-well flat plate, and several wells were pulsed with the hsp105 protein overnight, and the other wells were not pulsed therewith. To both types of wells, 30,000 Th cells were added, followed by the reaction for 72 hours (1 μCi of [^{3}H] thymidine was added to each well for the last 16 hours). Thereafter, the intake of [^{3}H] thymidine was measured with a liquid scintillation counter. The Th cells specifically reacted with the hsp105 protein and grew (Figure 6B). On the other hand, 24 hours after the addition of Th, the supernatant was kept. Thus, IFN-γ and IL-4 secreted from Th as a result of the reaction were measured with Mouse IFN-γ, IL-4 ELISA Ready-SET-Go! (eBioscience). The Th was of Th1 type, which generates a large amount of IFN-γ as a result of the specific reaction with the hsp105 protein (Figure 6C). Colon-26 was subcutaneously implanted into the back of a BALB/c mouse to form a tumor with a size of 3 mm. Thereafter, the above Th was injected into the local site, followed by observation of the progression. As a result, after such a treatment, the growth of the Colon-26 tumor was clearly retarded (Figure 6D).

From the above-described results, it was found that the BALB/c mouse CD4 positive helper T cell line induced by the hsp105 protein grows hsp105 protein-specifically, and that it delays the growth of a tumor mass of the colon cancer cell line Colon-26 that highly expresses hsp105.

Example 8: CD4 positive helper T cell line of colon cancer patient induced by hsp105

Monocytes were separated from the peripheral blood. 200,000 monocytes per well of a 96-well flat plate were cultured in 200 μl of a culture solution containing IL-2 (100 IU/ml) and a 2 μg/ml recombinant hsp105 protein for 1 week. Thereafter, every week, the culture product was repeatedly stimulated with 200,000 monocytes, which had been pulsed with a 2 μg/ml recombinant hsp105 protein and then exposed to radioactive rays, so as to establish multiple CD4 positive helper T cell lines (Th). The expression of CD4 and CD8 on the surface of cells was confirmed by performing immunofluorescent staining using Pharmingen anti-human CD4, CD8-FITC, and then analyzed with FACS. It was examined in the same manner as in Example 7, whether or not Th specifically reacts with the hsp105 protein and grows. The Th cells specifically

reacted with the hsp105 protein and grew (Figure 7A). In addition, in the same manner as in Example 7, IFN-γ and IL-4 secreted from Th as a result of the reaction were measured using Human IFN-γ, IL-4 US ELISA Kit (BIOSOURCE, Camarillo, CA). The Th was of Th1 type, which generates a large amount of IFN-γ as a result of the specific reaction with the hsp105 protein (Figure 7B). In general, it has been known that Th1 acts favorably for the induction of CTL and antitumor immunity. It was found that such Th1 can be induced also in humans by stimulating peripheral blood lympocytes with the hsp105 protein.

Example 9: *In vivo* antitumor activity of cytotoxic T lymphocytes stimulated with hsp105 peptide

It was examined whether or not BALB/c mouse cytotoxic T lymphocytes (CTL) induced by an hsp105-derived peptide Asn-Tyr-Gly-Ile-Tyr-Lys-Gln-Asp-Leu (SEQ ID NO: 3) reduce a tumor mass of the colon cancer cell line Colon-26 that highly expresses hsp105. Specifically, Colon-26 was subcutaneously implanted into the back of a BALB/c mouse to form a tumor with a size of 5 mm. Thereafter, CTL was injected into the local site. 1 week later, the mouse was subjected to anatomy, and the site was pathologically observed by HE staining. The results are shown in Figure 8. As is apparent from the results shown in Figure 8, the tumor was clearly reduced by administration of the CTL induced by the hsp105-derived peptide.

Also, it was examined whether or not the cytotoxic T lymphocytes (CTL) of a colon cancer patient induced by an hsp105-derived peptide Lys-Leu-Met-Ser-Ser-Asn-Ser-Thr-Asp-Leu (SEQ ID NO: 14) reduce a tumor mass of the colon cancer cell line sw620 that highly expresses hsp105. Specifically, sw620 was subcutaneously implanted into the back of a nude mouse to form a tumor with a size of 5 mm, and thereafter, CTL was injected into the local site.

1 week after injection of the CTL, the tumor was reduced. 2 weeks after the treatment, the mouse was subjected to anatomy, and the site was pathologically observed by HE staining. The results are shown in Figure 9. As is apparent from the results shown in Figure 9, the increase of the tumor was clearly retarded by administration of the CTL.

INDUSTRIAL APPLICABILITY

The cancer antigen protein and antigen peptide of the present invention, or DNA encoding the protein or peptide of the present invention, can be used as an excellent anti-cancer vaccine having few side effects such as self-injury. In addition, an antibody can be used as a diagnostic agent. Moreover, helper T cells, cytotoxic T lymphocytes, or an immunocyte population containing these cells, which are stimulated and activated with the antigen of the present invention, can be used as anticancer agents.